

Cecal Volatile Fatty Acids and Broiler Chick Susceptibility to *Salmonella typhimurium* Colonization as Affected by Aflatoxins and T-2 Toxin¹

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ABSTRACT Four experiments were conducted using day-of-hatch, mixed-sex broiler chicks to evaluate the effects of aflatoxins and T-2 toxin on cecal volatile fatty acids (VFA) and the susceptibility to *Salmonella* colonization. All chicks in these experiments were challenged orally with 10⁴ cfu of *Salmonella typhimurium* (ST) on Day 3. In Experiments 1 and 2, chicks were fed diets containing 0, 2.5, or 7.5 mg aflatoxins/kg of diet and were allowed to develop their microflora naturally. In Experiment 3, all chicks were orally gavaged on the day of hatch with a competitive exclusion (CE) culture (PREEMPT[™]) and were fed diets containing 0, 2.5, or 7.5 mg T-2 toxin/kg. In Experiment 4, the chicks were fed diets containing 0, 7.5, or 15.0 mg T-2 toxin/kg and one-half of the chicks were orally gavaged on the day of hatch with the CE culture. In Experiments 1 and 2, with the exception of increased total VFA at 5 d in chicks fed the 7.5 mg T-2 aflatoxins/kg diet, there were no treatment effects on cecal propionic acid, total VFA, or incidence or severity of ST colonization. In Experiment 3, the only alteration

in concentration of cecal propionic acid or total VFA was a significant reduction in total VFA at 5 d in chicks fed the 2.5 mg T-2 toxin/kg diet. No significant treatment differences were observed for numbers of *Salmonella* cecal culture-positive chicks or for numbers of ST in the cecal contents. In Experiment 4, with minor exceptions, the chicks treated with the CE culture had higher cecal concentrations of propionic acid and were less susceptible to *Salmonella* colonization than the non-CE-treated chicks. In the non-CE-treated chicks, T-2 toxin had no effect on any of the parameters, and 85 to 90% of the chicks were *Salmonella* cecal culture-positive. In the CE-treated chicks, there was a decrease in propionic acid concentration at 3 and 11 d and an increase in susceptibility to *Salmonella* colonization of the chicks fed the 15.0 mg T-2 toxin/kg diet. These results indicate that cecal concentrations of VFA can be affected by toxins, such as high concentrations of T-2 toxin, and that resistance to *Salmonella* colonization may be reduced. Further research is necessary to determine the biological significance of these changes.

(Key words: *Salmonella*, broiler chicks, volatile fatty acids, aflatoxins, T-2 toxin)

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INTRODUCTION

In recent years there has been increased consumer concern regarding the contamination of products with salmonellae. Poultry products are among those products frequently contaminated with various serotypes of *Salmonella*, and these products are a source of human salmonellosis (Cohen and Tauxe, 1986; Izat et al., 1991). Numerous factors can affect the susceptibility of chickens

to salmonellae colonization, including age, stress, general health, feed additives, the genetics of the chicken, and others (Bailey, 1988).

Newly hatched chicks are more susceptible to salmonellae colonization than older chicks that have developed resistance as native microflora become established. This increased susceptibility to salmonellae cecal colonization has been attributed to insufficient concentration of cecal volatile fatty acids (VFA) to prevent colonization (Barnes et al., 1979). Barnes et al. (1980) reported that cecal VFA concentrations are indicators of anaerobe growth. Nisbet et al. (1994, 1996) and Corrier et al. (1995) confirmed this hypothesis and demonstrated a correlation between cecal VFA concentrations, especially propionic acid, in 3-d-old

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Abbreviation Key: AF = aflatoxins; CE = competitive exclusion; ST = *Salmonella typhimurium*; VFA = volatile fatty acids.

TABLE 1. Effect of dietary aflatoxins (AF) on the concentrations of propionic acid and total volatile fatty acids in the cecal contents of broiler chicks at 3, 5, 7, 9, and 11 d of age (Experiment 1)¹

Treatment	Time									
	Propionic acid ($\mu\text{mol/g}$ cecal content)					Total volatile fatty acids ($\mu\text{mol/g}$ cecal content)				
	3 d	5 d	7 d	9 d	11 d	3 d	5 d	7 d	9 d	11 d
Control	2.84 ^a	3.06 ^a	4.92 ^a	4.54 ^a	5.91 ^a	26.14 ^a	41.24 ^a	82.00 ^a	72.86 ^a	94.73 ^a
2.5 mg AF/kg diet	2.76 ^a	3.26 ^a	4.90 ^a	4.12 ^a	4.20 ^a	25.75 ^a	49.20 ^a	88.10 ^a	68.53 ^a	88.50 ^a
7.5 mg AF/kg diet	2.69 ^a	3.02 ^a	4.75 ^a	4.11 ^a	4.95 ^a	29.99 ^a	32.81 ^a	78.27 ^a	63.46 ^a	83.74 ^a
LSD ²	0.26	0.34	0.90	0.92	2.13	17.29	24.00	15.15	13.60	17.23

^aMeans within a column with different superscripts differ significantly ($P < 0.05$).

¹Values represent the mean of 12 chicks per treatment (four replicates of three chicks each) for each sampling period.

²LSD = Least significant difference as determined by Fisher's protected LSD procedure.

chicks with the establishment of anaerobic cecal microflora and protection against *Salmonella typhimurium* (ST) colonization.

Adequate information is not available on the effects of specific dietary constituents or toxins on the native microflora or competitive exclusion (CE) cultures and salmonellae colonization. Mycotoxins are a group of fungal metabolites that are often present in grains consumed by humans or animals. Aflatoxins (AF), a group of extremely toxic chemicals, are produced by certain species of fungi in the genus *Aspergillus* and have been detected as contaminants of crops before harvest, between harvest and drying, in storage, and after processing and manufacturing (Council for Agricultural Science and Technology, 1989). Aflatoxins cause severe economic losses in the poultry and livestock industries. In many cases, AF contamination may mean the difference between profit and loss to the poultry industry (Jones et al., 1982; Nichols, 1983; Hamilton, 1984). Experimentally induced toxicity in young growing chicks has been well documented, as indicated by Huff et al. (1988). The T-2 toxin is a naturally occurring mycotoxin produced by several species of fungi in the genus *Fusarium* (Bamburg et al., 1970) that are found in many grains and feeds used in poultry diets. The T-2 toxin causes reduced performance and severe oral lesions in poultry (Wyatt et al., 1972, 1973b; Chi et al., 1977; Chi and Mirocha, 1978; Hoerr et al., 1981; Huff et al., 1988; Kubena et al., 1989, 1990, 1994), abnormal behavior (Wyatt et al., 1973a), altered feathering (Wyatt et al., 1975), and coagulopathy (Doerr et al., 1981). A significant interaction, resulting in increased mortality in chickens fed T-2 toxin and infected with several species of *Salmonella*, was observed by Boonchuvit et al. (1975). These workers suggested that the intestinal pathogens may be able to invade at necrotic sites in the gastrointestinal tract mucosa caused by the T-2 toxin. Corrier et al. (1989) observed a significant increase in numbers of chickens that were cecal culture-positive for ST when treated with T-2 toxin. Ziprin and Elissalde (1990) found a negative effect of T-2 toxin on the ability of chickens to resist salmonellosis, as measured by survival. These researchers did not determine the effects of T-2 toxin on the cecal propionic acid and total VFA concentrations.

Because of the widespread occurrence of the AF and T-2 toxin in grains and the likelihood of using grains contaminated with one of these mycotoxins in poultry feed, it is important to know whether these mycotoxins affect the establishment of microflora in young chicks, thus affecting VFA and susceptibility to enteropathogens such as salmonellae.

The purpose of the present research was to evaluate the effects of AF and T-2 toxin on cecal propionic acid and total VFA concentrations in young chicks with native microflora and chicks administered a commercial, characterized CE culture and on ST colonization of ceca.

MATERIALS AND METHODS

Four experiments were conducted using day-of-hatch, mixed-sex broiler chicks (Arbor Acres \times Arbor Acres) obtained from a commercial hatchery. The chicks were maintained in electrically heated batteries under continuous fluorescent lighting with feed and water provided ad libitum. The diets were formulated without added antibiotics, coccidiostats, or growth promoters and contained or exceeded the levels of nutrients recommended by the National Research Council (1994). The paper liners from the chick transport boxes were cultured successively in buffered peptone water in selenite cystine broth and on BGA plates as described previously (Andrews et al., 1978) and were examined for salmonellae. *Salmonella* spp. were not detected in the paper liners.

TABLE 2. Effect of dietary aflatoxins (AF) on the number of broiler chicks that were cecal culture-positive for *Salmonella typhimurium* and the number of *Salmonella typhimurium* in the cecal contents of 11-d-old broiler chicks (Experiment 1)

Treatment	<i>Salmonella</i> culture-positive chicks/total (%)	Log_{10} <i>Salmonella</i> per g cecal contents (number of chicks)
Control	15/20 (75)	4.35 (20)
2.5 mg AF/kg diet	13/20 (65)	3.67 (20)
7.5 mg AF/kg diet	15/20 (75)	4.22 (20)
LSD ¹		1.80

¹LSD = Least significant difference as determined by Fisher's protected LSD procedure.

TABLE 3. Effect of dietary aflatoxins (AF) on the concentrations of propionic acid and total volatile fatty acids in the cecal contents of broiler chicks at 3, 5, 8, and 10 d of age (Experiment 2)¹

Treatment	Time							
	Propionic acid ($\mu\text{mol/g}$ cecal content)				Total volatile fatty acids ($\mu\text{mol/g}$ cecal content)			
	3 d	5 d	8 d	10 d	3 d	5 d	7 d	10 d
Control	ND	2.95 ^a	3.03 ^a	3.31 ^a	15.64 ^a	22.99 ^b	50.14 ^{ab}	84.31 ^a
2.5 mg AF/kg diet	ND	2.76 ^a	2.93 ^a	3.09 ^a	21.65 ^a	35.54 ^{ab}	64.54 ^a	88.61 ^a
7.5 mg AF/kg diet	ND	2.73 ^a	2.97 ^a	3.47 ^a	17.97 ^a	45.06 ^a	44.00 ^b	71.21 ^a
LSD ²		0.23	0.46	0.62	13.52	13.96	19.14	27.44

^{a,b}Means within a column with different superscripts differ significantly ($P < 0.05$).

¹Values represent the mean of 12 chicks per treatment (four replicates of three chicks each) for each sampling period.

²LSD = Least significant difference as determined by Fisher's protected LSD procedure.

In all experiments, chicks were randomly assigned to groups of 80 chicks each (four replicates of 20 chicks each). All chicks were challenged by crop gavage at 3 d of age with 10^4 cfu ST, because previous research (D. E. Corrier, unpublished data) indicated that the challenge with ST did not result in a change in cecal VFA content. At 3, 5, 7, and 9 d (or slight variations thereof) of age, 12 chicks per treatment group (four replicates of three chicks each) were killed by cervical dislocation, and cecal contents from each chick were collected aseptically. The concentrations of propionic acid and total VFA (acetic, propionic, butyric, isobutyric, valeric, and isovaleric acids) in the cecal contents were determined by gas liquid chromatography as described by Corrier et al. (1990). On the final day of the experiment, 0.25-g samples of the contents of one cecum from each of 12 chicks per treatment group were serially diluted and spread-plated on BGA plates at dilutions of 1:10, 1:100, 1:1000, and 1:10,000. The plates were incubated for 24 h at 37 C, and the number of colony-forming units of ST per gram of cecal contents was determined on an automatic colony counter.⁴ Typical *Salmonella* colonies were confirmed by biochemical tests on triple sugar agar and lysine iron agar⁵ and further identified as ST serologically using *Salmonella* O antiserum, Group 13, Factors 1, 4, 12, and 15.⁶ *Salmonella* colony plate counts were expressed as \log_{10} *Salmonella* per gram of cecal contents. Cecal contents that were *Salmonella* culture negative at the 1:100 dilution but positive after culture in selenite-cystine were arbitrarily assigned a value of 1.50 \log_{10} *Salmonella* per gram of cecal contents. Selenite-cystine cultures that were negative on BGA plates were assigned a \log_{10} *Salmonella* value of 0 (Corrier et al., 1993, 1995; Byrd et al., 1998).

In Experiments 1 and 2, chicks were allowed to develop their microflora naturally and were fed diets containing 0 (controls), 2.5, or 7.5 mg AF/kg of diet. In Experiment

3, the chicks were orally gavaged at 1 d of age with a CE culture (PREEMPTTM),⁷ a commercial CE product, according to manufacturer's recommendations. This product, which was developed in our laboratory and licensed to the company, contains 29 characterized bacterial isolates (Corrier et al., 1995) and is the only CE product approved for use against *Salmonella* in poultry in the United States at the present time. The chicks were fed diets containing 0 (controls), 2.5, or 7.5 mg T-2 toxin⁶/kg for 9 d. In Experiment 4, the chicks were maintained under conditions as described in Experiment 3. The chicks were randomly assigned to six groups of 80 chicks each (four replicates of 20 chicks each). Two groups of chicks (eight replicates of 20 chicks each) were fed diets containing 0 (controls), 7.5, or 15.0 mg T-2 toxin/kg for 11 d. Three groups of chicks (one group from each dietary treatment) were orally gavaged with the CE culture as previously described for Experiment 3 and were maintained in a separate room under similar conditions. Sampling of chicks at 3, 5, 8, and 11 d was performed as previously described. Upon termination of Experiment 4, all remaining chicks were individually weighed.

The AF for the experiment was produced through the fermentation of rice by *Aspergillus parasiticus* NRRL 2999 by methods previously described by Kubena et al. (1990). The AF content was measured by spectrophotometric analysis (Nabney and Nesbitt, 1965), as modified by Wiseman et al. (1967). The AF within the rice powder comprised 79% AFB₁, 16% AFG₁, 4% AFB₂, and 1% AFG₂. The

TABLE 4. Effect of dietary aflatoxins (AF) on the number of broiler chicks that were cecal culture-positive for *Salmonella typhimurium* and the number of *Salmonella typhimurium* in the cecal contents of 10-d-old broiler chicks (Experiment 2)

Treatment	<i>Salmonella</i> culture-positive chicks/total (%)	\log_{10} <i>Salmonella</i> per g cecal contents (number of chicks)
Control	17/20 (85)	5.30 (20)
2.5 mg AF/kg diet	18/20 (90)	5.50 (20)
7.5 mg AF/kg diet	20/20 (100)	6.11 (20)
LSD ¹		1.30

¹LSD = Least significant difference as determined by Fisher's protected LSD procedure.

⁴Biotran III, New Brunswick Scientific Co., Edison, NJ 08818-4004.

⁵Difco Laboratories, Detroit, MI 48232.

⁶Kindly provided by G. E. Rottinghaus, Veterinary Medical Diagnostic Laboratory, College of Veterinary Medicine, University of Missouri, Columbia, MO 65602.

⁷Bioscience Division of Milk Specialties Co., 3802 Packers Ave., Madison, WI 53704.

TABLE 5. Effect of dietary T-2 toxin on the concentrations of propionic acid and total volatile fatty acids in the cecal contents of broiler chicks at 3, 5, 7, and 9 d of age (Experiment 3)¹

Treatment	Time							
	Propionic acid ($\mu\text{mol/g}$ cecal content)				Total volatile fatty acids ($\mu\text{mol/g}$ cecal content)			
	3 d	5 d	7 d	9 d	3 d	5 d	7 d	9 d
Control	28.71 ^a	25.42 ^{ab}	26.62 ^a	33.88 ^a	71.89 ^a	87.80 ^a	94.42 ^a	118.76 ^a
2.5 mg T-2 toxin/kg diet	23.29 ^a	18.23 ^b	28.02 ^a	46.30 ^a	62.29 ^a	58.80 ^b	80.90 ^a	125.88 ^a
7.5 mg T-2 toxin/kg diet	26.97 ^a	31.30 ^a	25.16 ^a	38.17 ^a	65.04 ^a	86.86 ^a	90.83 ^a	115.22 ^a
LSD ²	7.30	7.80	9.84	17.28	11.33	14.37	26.99	36.62

^{a,b}Means within a column with different superscripts differ significantly ($P < 0.05$).

¹Values represent the mean of 12 chicks per treatment (four replicates of three chicks each) for each sampling period.

²LSD = Least significant difference as determined by Fisher's protected LSD procedure.

basal diet was analyzed for mycotoxins and was found to be below detection limits ($<10 \mu\text{g/kg}$) for AF, deoxynivalenol, zearalenone, ochratoxin, and cyclopiazonic acid by the methods described by Clement and Phillips (1985).

By nuclear magnetic resonance and mass spectrometry, the T-2 was determined to be greater than 99% pure. The T-2 toxin was incorporated into the diet by dissolving it in 95% ethanol and then mixing the appropriate quantities with 1 kg of the diet. After drying, the 1-kg quantities of diet were mixed with the rest of the basal diet to produce the treatments containing T-2 toxin. Data for body weights (Experiment 4 only), individual VFA, total VFA, and \log_{10} cfu counts among treatment groups were subjected to ANOVA (Snedecor and Cochran, 1967) using the general linear models procedure in the PC-SAS[®] statistical software (SAS Institute, 1987). Variable means showing significant differences in the ANOVA were compared using the Fisher's protected least significant difference (LSD) procedure (Snedecor and Cochran, 1967). Differences in the number of *S. typhimurium* culture-positive chicks between treatment groups were analyzed by chi-squared analysis (Luginbue and Schlotzhauer, 1987). All statements of significance were based on the 0.05 level of probability, unless otherwise stated.

RESULTS

In Experiments 1 and 2, feeding chicks diets containing 2.5 or 7.5 mg AF/kg of diet resulted in no significant changes in cecal propionic acid or total VFA concentrations, with the exception at 5 d, when chicks fed the 7.5 mg/kg diet had significantly higher concentrations of total VFA than the controls (Tables 1 and 3). There were no significant treatment differences observed for numbers of chicks with *Salmonella*-positive cecal cultures or in numbers of ST in the cecal contents (Tables 2 and 4).

Compared with controls, in Experiment 3 the only alteration in concentrations of cecal propionic acid and total VFA was a significant reduction in total VFA at 5 d in chicks fed the 2.5 mg T-2 toxin/kg diet (Table 5). There were no significant treatment differences observed for numbers of chicks with *Salmonella*-positive cecal cultures or in numbers of ST in the cecal contents (Table 6).

The data for concentrations of cecal propionic acid and total VFA in Experiment 4 are presented in Table 7. With

minor exceptions, the propionic acid concentrations were significantly higher in chicks treated with the CE culture than the non-CE-treated chicks at 3, 5, 8, and 11 d. Total cecal VFA concentrations tended to be higher in CE-treated chicks when compared with non-CE-treated chicks; however, this finding was statistically significant only for control CE-treated chicks at Days 3 and 11 and at Day 11 for the CE-treated chicks fed the 7.5 mg T-2 toxin/kg diet. Within the non-CE-treated group of chicks, with the exception of Day 8 when the chicks fed the 7.5 mg T-2 toxin/kg diet had higher propionic acid concentrations, there were no significant differences in cecal concentrations of propionic acid or total VFA due to T-2 toxin. Within the CE-treated chicks, there was a significant decrease in cecal propionic acid concentrations at 3 and 11 d in the chicks fed the 15.0 mg T-2 toxin/kg diet and no changes in the concentration of total VFA when compared to the control CE-treated chicks.

Body weights at Day 11 were significantly lower for chicks fed the 15.0 mg T-2 toxin/kg diet with or without CE culture (Table 8). The experimental challenge dose of 10^4 cfu ST resulted in cecal colonization in 85% of the non-CE-treated control chicks. There was no difference due to T-2 toxin treatment. This challenge resulted in a significant ($P < 0.001$) decrease in cecal colonization to only 10% of the CE-treated control chicks. There was a significant ($P < 0.05$) increase in the number of chicks with *Salmonella*-positive cecal cultures fed the 15.0 mg T-2 toxin/kg diet, when compared with CE-treated controls (45 vs. 10%). Cecal colonization in the chicks fed the 7.5

TABLE 6. Effect of dietary T-2 toxin on the number of broiler chicks that were cecal culture-positive for *Salmonella typhimurium* and the number of *Salmonella typhimurium* in the cecal contents of 9-d-old broiler chicks (Experiment 3)

Treatment	<i>Salmonella</i> culture-positive chicks/total (%)	\log_{10} <i>Salmonella</i> per g cecal contents (number of chicks)
Controls	1/20 (5)	0.19 (20)
2.5 mg T-2 toxin/kg diet	5/20 (25)	0.51 (20)
7.5 mg T-2 toxin/kg diet	1/20 (5)	0.23 (25)
LSD ¹		0.63

¹LSD = Least significant difference as determined by Fisher's protected LSD procedure.

TABLE 7. Effect of dietary T-2 toxin and competitive exclusion (CE) culture¹ on the concentrations of propionic acid and total volatile fatty acids in the cecal contents of broiler chicks at 3, 5, 8, and 11 d of age (Experiment 4)²

Treatment	Time							
	Propionic acid ($\mu\text{mol/g}$ cecal content)				Total volatile fatty acids ($\mu\text{mol/g}$ cecal content)			
	3 d	5 d	8 d	11 d	3 d	5 d	8 d	11 d
Control	0.54 ^c	1.92 ^b	2.69 ^d	6.88 ^c	18.25 ^b	37.88 ^a	60.13 ^a	77.17 ^b
7.5 mg T-2 toxin/kg diet	1.22 ^c	1.36 ^b	5.18 ^{bc}	6.34 ^c	20.98 ^{ab}	30.17 ^a	51.56 ^a	74.28 ^b
15.0 mg T-2 toxin/kg diet	0.54 ^c	1.54 ^b	3.89 ^{cd}	6.19 ^c	17.08 ^b	31.57 ^a	50.67 ^a	77.63 ^b
Control + CE culture	7.58 ^a	5.76 ^a	7.28 ^{ab}	17.70 ^a	29.22 ^a	41.72 ^a	54.18 ^a	107.22 ^a
7.5 mg T-2 + CE culture	5.41 ^{ab}	7.36 ^a	7.47 ^{ab}	13.96 ^{ab}	23.09 ^{ab}	41.79 ^a	61.43 ^a	108.21 ^a
15.0 mg T-2 + CE culture	5.01 ^b	6.31 ^a	8.21 ^a	11.82 ^{bc}	24.77 ^{ab}	33.97 ^a	46.68 ^a	95.03 ^{ab}
LSD ³	2.53	1.94	2.65	5.06	9.22	14.05	17.46	23.25

^{a-c}Means within a column with no different superscripts differ significantly ($P < 0.05$).

¹The CE culture was PREEMPT™, Bioscience Division of Milk Specialties Co., 3802 Packers Ave., Madison, WI 53507.

²Values represent the mean of 12 chicks per treatment (four replicates of three chicks each) for each sampling period.

³LSD = Least significant difference as determined by Fisher's protected LSD procedure.

mg T-2 toxin/kg diet was intermediate at 35%, which was not significantly different from the CE-treated control chicks. Compared with non-CE-treated controls, the numbers of ST in the cecal contents of the CE-treated controls decreased ($P < 0.05$) by 3.82 \log_{10} units. There were no significant differences in the numbers of ST in the cecal contents due to dietary T-2 toxin, although the values appeared to increase numerically in the chicks fed the T-2 toxin diets.

DISCUSSION

Compared with controls, feeding broiler chicks 2.5 or 7.5 mg AF/kg of diet did not significantly alter the concentrations of cecal propionic acid or total VFA. In Experiment 1, the challenge dose of 10^4 cfu ST resulted in *Salmonella*-positive cecal cultures at the end of the experiment in 75% of the control chicks, 65% of the chicks fed 2.5 mg AF/kg, and 75% of the chicks fed the 7.5 mg AF/kg. The \log_{10} *Salmonella* per gram of cecal contents for these treatments were 4.35, 3.67, and 4.22 for the treatments, respectively. In Experiment 2, these treatments had 85,

90, and 100% chicks with *Salmonella*-positive cultures with \log_{10} *Salmonella* per gram of cecal contents of 5.30, 5.5, and 6.11, respectively. These data indicate that feeding AF at 2.5 or 7.5 mg/kg did not significantly affect any of the parameters measured. This information is important because during years of drought or other stressors, grains used in poultry diets may very likely be contaminated with AF.

Feeding young chicks 7.5 or 15.0 mg T-2 toxin/kg of diet for 11 d resulted in no change in body weights at 7.5 mg T-2 toxin/kg and a significant reduction at 15.0 mg T-2 toxin/kg of diet (Experiment 4). The concentration of 7.5 mg T-2 toxin/kg of diet is a borderline concentration for observing reductions in body weights at this early age under laboratory conditions (Kubena et al., 1994).

Propionic acid and total VFA concentrations were consistently high throughout Experiment 3 because all chicks were administered CE culture. The only significant ($P > 0.05$) effect observed was a decrease in total VFA at 5 d in chicks fed the 2.5 mg T-2 toxin/kg diet. Interestingly, although not statistically significant, this treatment also had the numerically highest incidence of chicks with *Sal-*

TABLE 8. Effect of dietary T-2 toxin and competitive exclusion (CE) culture¹ on body weights, the number of chicks cecal culture-positive for *Salmonella typhimurium*, and the number of *Salmonella typhimurium* in the cecal contents of 11-d-old broiler chicks (Experiment 4)

Treatment	Body weight (number) g	<i>Salmonella</i> culture-positive chicks/total (%)	\log_{10} <i>Salmonella</i> per g cecal contents (number of chicks)
Control	221 ^a (25)	17/20 (85)	4.54 ^a (20)
7.5 mg T-2 toxin/kg diet	217 ^a (28)	18/20 (90)	5.34 ^a (20)
15.0 mg T-2 toxin/kg diet	169 ^b (31)	17/20 (85)	5.18 ^a (20)
Control + CE culture	225 ^a (28)	52/60 (87)	0.62 ^b (20)
7.5 mg T-2 + CE culture	212 ^a (28)	2/20 (10)	1.48 ^b (20)
15.0 mg T-2 + CE culture	177 ^b (28)	7/20 (35)	2.09 ^b (20)
LSD ²	15	9/20 (45)*	LSD 1.60
		18/60 (30)**	

^{a,b}Means within a column with different superscript differ significantly ($P < 0.05$).

¹The CE culture was PREEMPT™, Bioscience Division of Milk Specialties Co., 3802 Packers Ave., Madison, WI 53704.

²LSD = Least significant difference as determined by Fisher's protected LSD procedure.

*Significantly different from Control + CE culture-treated ($P < 0.05$).

**Significantly different from combined non-CE culture-treated chicks ($P < 0.01$).

monella-positive cecal cultures, when compared with controls (5/20 vs. 1/20).

In Experiment 4, body weights at 11 d were significantly reduced ($P < 0.05$) in chicks fed the 15.0 mg T-2 toxin/kg diet in both CE-treated and untreated chicks, indicating overt toxicity due to the highest concentration of T-2 toxin. In the non-CE-treated chicks, feeding the 7.5 or 15.0 mg T-2 toxin/kg of diet did not significantly affect the concentrations of propionic acid, total VFA, the numbers of *Salmonella* culture-positive chicks or the numbers of *Salmonella* per gram of cecal contents. In the CE-treated chicks, the chicks fed 15.0 mg T-2 toxin/kg diet had significantly lower concentrations of cecal propionic acid at 3 and 11 d, but there were no differences in total VFA.

The expected increase in propionic acid concentrations detected in chicks administered the CE culture is a characteristic indication of the establishment of the culture in the digestive tract (Nisbet et al., 1994; Corrier et al., 1995; Droleskey et al., 1995). The reason for the lower concentrations of cecal propionic acid, when compared with Experiment 3 and some other reports, is unknown. Perhaps in this experiment a different microbial population was present before the chicks were administered the CE culture. However, there was a 14-fold increase in the concentration of propionic acid in the CE-treated controls compared with the non-CE-treated controls.

Eighty-five to ninety percent of the non-CE-treated chicks had *Salmonella*-positive cecal cultures, and the \log_{10} *Salmonella* per gram of cecal contents ranged from 4.54 to 5.34. Treatment of the chicks at day of hatch with the CE culture resulted in a decrease ($P < 0.001$) in chicks with *Salmonella*-positive cecal cultures (30 vs. 87%) and a significant decrease ($P < 0.05$) in the number of *Salmonella* per gram of cecal contents (\log_{10} 5.03 vs. 1.40). Interestingly, chicks with 7.5 μmol cecal propionic acid per gram of cecal contents were protected to a greater extent from cecal colonization by *Salmonella* when compared to chicks with slightly lower concentrations of propionic acid (5.41 and 5.01). These results agree with the results of Nisbet et al. (1996), who observed significant protection with 7.5 μmol propionic acid per gram of cecal contents. However, in the present research, 5.4 and 5.1 μmol propionic acid per gram of cecal contents also showed significant protection against colonization, whereas Nisbet et al. (1996) did not report protection at 2.1 to 5.4 μmol propionic acid. It is unlikely that this increase in propionic acid concentration is solely responsible for the decrease in colonization when compared with the other two CE culture treatments, but could be due to other factors such as competition for limiting nutrient(s) required for optimal propionic acid formation (Nisbet et al., 1996). Ha et al. (1995) demonstrated that competition for serine in an anaerobic environment was an important factor in a coculture containing ST and a native cecal bacterial isolate. Although it is not well understood how propionic acid functions in the mechanism(s) in the reduction of *Salmonella* cecal colonization, the present research supports the previous work of Corrier et al. (1995) and Nisbet et al. (1994, 1996) showing it is a biological indicator

correlated with *Salmonella* control. Another possibility is that the T-2 toxin may decrease host resistance to *Salmonella* by the well-demonstrated inflammatory and irritant action of T-2 toxin toward the gastrointestinal tract (Marasas et al., 1969; Wyatt et al., 1973b), which suggests that the natural barriers to invasion by *Salmonella* may be more easily broken during T-2 toxicosis (Perry et al., 1972). Impaired immune function may also be a factor (Corrier et al., 1989; Ziprin and Elissalde, 1990). Another factor that may have a role is the generalized stress caused by T-2 toxin, as indicated by decreases in the weight of the bursa of Fabricius (Boonchuvit et al., 1975; Corrier et al., 1989).

Results of the present research indicate that the concentration of propionic acid produced in the ceca of young chicks may be an important part of the mechanism(s) that inhibit ST colonization of young chicks by anaerobic bacteria. These results also indicate that cecal concentrations of VFA can be affected by dietary toxins, such as high concentrations of the mycotoxin, T-2. Further research is needed to determine the biological significance of these changes.

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REFERENCES

- Andrews, W. H., P. L. Poelma, C. R. Wilson, and A. Romero, 1978. Isolation and identification of *Salmonella*. Pages 1–24 in: Bacteriological Analytical Manual. 5th ed. Association of Official Analytical Chemists, Washington, DC.
- Bailey, J. S., 1988. Integrated colonization control of *Salmonella* in poultry. Poultry Sci. 67:928–932.
- Bamburg, J. R., F. M. Strong, and E. B. Smalley, 1970. Toxins from moldy feed cereals. J. Agric. Food Chem. 17:443–450.
- Barnes, E. M., C. S. Impey, and D. M. Cooper, 1980. Manipulation of the crop and intestinal flora of the newly hatched chick. Am. J. Clin. Nutr. 33:2425–2433.
- Barnes, E. M., C. S. Impey, and B. J. H. Stevens, 1979. Factors affecting the incidence and anti-*Salmonella* activity of the anaerobic cecal flora of the chick. J. Hyg. 82:263–283.
- Boonchuvit, B., P. B. Hamilton, and H. R. Burmeister, 1975. Interaction of T-2 toxin with *Salmonella* infections of chickens. Poultry Sci. 54:1693–1696.
- Byrd, J. A., D. E. Corrier, J. R. DeLoach, D. J. Nisbet, and L. H. Stanker, 1998. Horizontal transmission of *Salmonella typhimurium* in broiler chicks. J. Appl. Poultry Res. 7:75–80.
- Chi, M. S., and C. J. Mirocha, 1978. Necrotic oral lesions in chickens fed diacetoxyscirpenol, T-2 toxin and crocotoxin. Poultry Sci. 57:807–808.
- Chi, M. S., C. J. Mirocha, H. J. Kurtz, G. Weaver, F. Bates, and W. Shimoda, 1977. Subacute toxicity of T-2 toxin in broiler chicks. Poultry Sci. 56:306–313.
- Clement, B. A., and T. D. Phillips, 1985. Advances in the detection and determination of mycotoxins via capillary GC/quadrupole mass spectrometry. Toxicologist 5(1):232. (Abstr.).

- Cohen, M. L., and R. V. Tauxe, 1986. Drug-resistance *Salmonella* in the United States: an epidemiologic perspective. *Science* 234:964-969.
- Corrier, D. E., J. R. DeLoach, and R. L. Ziprin, 1989. Effect of T-2 toxin, cyclophosphamide, or deoxamethasone on the resistance of young chickens to *Salmonella* colonization. *Poultry Sci.* 68(Suppl. 1):34. (Abstr.).
- Corrier, D. E., A. Hinton, Jr., R. L. Ziprin, and J. R. DeLoach, 1990. Effect of dietary lactose on *Salmonella* colonization of market-age broiler chickens. *Avian Dis.* 34:668-676.
- Corrier, D. E., D. J. Nisbet, A. G. Hollister, C. M. Scanlan, B. M. Hargis, and J. R. DeLoach, 1993. Development of defined cultures and indigenous cecal bacteria to control salmonellosis in broiler chicks. *Poultry Sci.* 72:1164-1168.
- Corrier, D. E., D. J. Nisbet, C. M. Scanlan, A. G. Hollister, and J. R. DeLoach, 1995. Control of *Salmonella typhimurium* colonization in broiler chicks with a continuous-flow characterized mixed culture of bacteria. *Poultry Sci.* 74:916-924.
- Council for Agricultural Science and Technology, 1989. Pages 1-91 in: *Mycotoxins: Economic and Health Risks*. K. A. Nisi, ed. Council for Agricultural Science and Technology, Ames, IA.
- Doerr, J. A., P. B. Hamilton, and H. R. Burmeister, 1981. T-2 toxicosis and blood coagulation in young chickens. *Toxicol. Appl. Pharmacol.* 60:157-162.
- Droleskey, R. E., D. E. Corrier, D. J. Nisbet, and J. R. DeLoach, 1995. Colonization of cecal mucosal epithelium in chicks treated with a continuous flow culture of 29 characterized bacteria: Confirmation by scanning electron microscopy. *J. Food Prot.* 58:837-842.
- Ha, S. A., S. C., Ricke, D. J. Nisbet, D. E. Corrier, and J. R. DeLoach, 1995. Serine utilization as a potential competition mechanism between *Salmonella typhimurium* and a chicken cecal bacterium. *J. Food Prot.* 57:1074-1079.
- Hamilton, P. B., 1984. Determining safe levels of mycotoxins. *J. Food Prot.* 47(7):570-575.
- Hoerr, F. J., W. W. Carlton, and B. Yagen, 1981. The toxicity of T-2 toxin and diacetoxyscirpenol in combination for broiler chickens. *Food Cosmetics Toxicol.* 19:185-188.
- Huff, W. E., R. B. Harvey, L. F. Kubena, and G. E. Rottinghaus, 1988. Toxic synergism between aflatoxin and T-2 toxin in broiler chickens. *Poultry Sci.* 67:1418-1423.
- Izat, A. L., J. M. Kopek, and J. D. McGinnis, 1991. Research notes: incidence, number, and serotypes of *Salmonella* on frozen broiler chickens at retail. *Poultry Sci.* 70:1438-1440.
- Jones, F. T., W. M. Hagler, and P. B. Hamilton, 1982. Association of low levels of aflatoxin in feed with productivity losses in commercial broiler operations. *Poultry Sci.* 61:861-868.
- Kubena, L. F., R. B. Harvey, W. E. Huff, D. E. Corrier, T. D. Phillips, and G. E. Rottinghaus, 1989. Influence of ochratoxin A and T-2 toxin singly and in combination on broiler chickens. *Poultry Sci.* 68:867-872.
- Kubena, L. F., R. B. Harvey, W. E. Huff, D. E. Corrier, T. D. Phillips, and G. E. Rottinghaus, 1990. Efficacy of a hydrated sodium calcium aluminosilicate to reduce the toxicity of aflatoxin and T-2 toxin. *Poultry Sci.* 69:1078-1086.
- Kubena, L. F., E. E. Smith, A. Gentles, R. B. Harvey, T. E. Edrington, T. D. Phillips, and G. E. Rottinghaus, 1994. Individual and combined toxicity of T-2 toxin and cyclopiazonic acid in broiler chicks. *Poultry Sci.* 73:1390-1397.
- Luginbuke, R. C., and S. D. Schlotzhauer, 1987. Pages 555-573 in: *SAS/STAT® Guide for Personal Computers*, 6th ed. SAS Institute Inc., Cary, NC.
- Marasas, W.F.O., J. R. Bamburg, E. B. Smalley, F. M. Strong, W. L. Ragland, and P. E. Degurse, 1969. Toxic effects on trout, rats, and mice of T-2 toxin produced by the fungus *Fusarium tricinatum* (Cd.) Synd. Hans. *Toxicol. Appl. Pharmacol.* 15:471-482.
- Nabney, J., and B. F. Nesbitt, 1965. A spectrophotometric method of determining the aflatoxins. *Analyst* 90:155-160.
- National Research Council, 1994. *Nutrient Requirements of Poultry*. 9th rev. ed. National Academy Press, Washington, DC.
- Nichols, T. E., 1983. Economic effects of aflatoxin in corn. Pages 67-71 in: *Aflatoxin and Aspergillus flavus* in corn. U. Diener, R. Asquith, and J. Dickens, ed. Soothern Coop. Ser. Bulletin 279. Auburn University, Auburn, AL.
- Nisbet, D. J., D. E. Corrier, S. C. Ricke, M. E. Hume, J. A. Byrd II, and J. R. DeLoach, 1996. Cecal propionic acid as a biological indicator of the early establishment of a microbial ecosystem inhibitory to *Salmonella* in chicks. *Anaerobe* 2:345-350.
- Nisbet, D. J., S. C. Ricke, C. M. Scanlan, D. E. Corrier, A. G. Hollister, and J. R. DeLoach, 1994. Inoculation of broiler chicks with a continuous-flow derived bacterial culture facilitates early cecal bacterial colonization and increases resistance to *Salmonella typhimurium*. *J. Food Prot.* 57:12-15.
- Perry, S. C., T. J. Hebert, and A. C. Braemer, 1972. The effect of furazolidone on artificially induced *Salmonella typhimurium* and *Salmonella gallinarum* infection in chickens. *Poultry Sci.* 51:906-912.
- SAS Institute, 1987. *SAS/STAT® Guide for Personal Computers*. Version 6.02. 6th ed. SAS Institute Inc., Cary, NC.
- Snedecor, G. W., and W. G. Cochran, 1967. Pages 258-380 in: *Statistical Methods*. 6th ed. The Iowa State University Press, Ames, IA.
- Wiseman, H. G., W. C. Jacobson, and W. E. Harmeyer, 1967. Note on the removal of pigments from chloroform extracts of aflatoxin cultures with copper carbonate. *J. Assoc. Off. Agric. Chem.* 50:982-983.
- Wyatt, R. D., W. M. Colwell, P. B. Hamilton, and H. R. Burmeister, 1973a. Neural disturbances in chickens caused by dietary T-2 toxin. *Appl. Microbiol.* 26:757-761.
- Wyatt, R. D., P. B. Hamilton, and H. R. Burmeister, 1973b. The effects of T-2 toxin in broiler chickens. *Poultry Sci.* 52:1853-1859.
- Wyatt, R. D., P. B. Hamilton, and H. R. Burmeister, 1975. Altered feathering of chicks caused by T-2 toxin. *Poultry Sci.* 54:1042-1045.
- Wyatt, R. D., B. A. Weeks, P. B. Hamilton, and H. R. Burmeister, 1972. Severe oral lesions in chickens caused by ingestion of dietary fusariotoxin T-2. *Appl. Microbiol.* 24:251-257.
- Ziprin, R. L., and M. H. Elissalde, 1990. Effect of T-2 toxin on resistance to systemic *Salmonella typhimurium* infection of newly hatched chickens. *Am. J. Vet. Res.* 51:1869-1872.